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Further studies of natural killer cell function in Chediak-Higashi patients

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Summary. Spontaneous natural killer (NK) activity and antibody-dependent cellular cytotoxicity (ADCC) of blood lymphocytes against five human tumour cell lines (K562, Molt-4, HL-60, Chang, Daudi) and three mouse tumour lines (YAC, P815, RBL-5) were ten- to 100-fold lower than normal in six patients with Chediak-Higashi (CH) disease. NK and ADCC were defective at 4 hr, and less so at 18 hr. The NK activity in normals and CH patients was mediated in part by FcR^+, E^- effector cells. ADCC against human erythrocytes was normal in CH patients, as were lectin-dependent cytolysis and mixed lymphocyte proliferative responses. Phagocytosis of antibody-coated ox erythrocytes was normal in CH patients as well. These observations confirm that the CH syndrome is associated with a profound and selective defect in NK and ADCC activity against tumour cells, whereas other mononuclear cell-mediated functions are normal.

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; CH, Chediak-Higashi; CTL, cytolytic T lymphocyte; E, receptor for sheep erythrocytes; EA, antibody-coated ox erythrocytes; FcR, receptor for the Fc portion of immunoglobulins; LU, lytic unit (the number of effector cells required to lyse a given percentage of target cells); MLC, mixed lymphocyte culture; NK, natural killer cell.

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INTRODUCTION

Humans with Chediak-Higashi (CH) syndrome develop a lymphoproliferative disorder which may be malignant (Dent, Fish, White & Good, 1966), and two prelymphomatous patients had markedly depressed levels of natural killer (NK) activity (Roder, Haliotis, Klein, Korec, Jett, Ortaldo, Herberman, Katz & Fauci, 1980). However, both of these patients were from the same family and were much older (28 and 29 years) than all other reported cases (Blume & Wolff, 1972). We therefore studied six CH patients in the prelymphomatous phase, one healthy sibling and appropriate normal donors for NK activity, antibody-dependent cellular cytotoxicity (ADCC), lectin-dependent cytotoxicity, mixed lymphocyte responses (MLC), and phagocytosis. These results confirm that CH patients have a selective NK deficiency.

MATERIALS AND METHODS

Blood donors

Blood was drawn in various centers from CH patients and normal adult controls and the mononuclear cell fraction was shipped to Kingston where it was usually received within 24–30 hr. Two on-site studies were done in Indianapolis and Bethesda. In both cases, the

use of fresh CH or normal lymphocytes yielded similar data indicating that the 24-hr shipment period did not adversely affect the cells.

Cell lines

All cell lines were maintained by continuous *in vitro* culture and were free of mycoplasma at the time of testing. The following cell lines were used: K562, derived from human myeloid leukaemia (erythroleukaemia); Molt-4, T-cell line derived from human acute lymphocytic leukaemia; Daudi, human B lymphoblastoid cell line; Chang, a cell line derived from human hepatocarcinoma; HL-60, myelogenous leukaemia line (promyelocytic); P815, methylcholanthrene-induced murine mastocytoma; P815-2, a subline of P815; YAC, a Moloney-virus-induced, murine T-cell-lymphoma line; RBL-5, a Rad-LV-induced murine lymphoma line.

Lymphocyte preparation

Heparinized venous blood was centrifuged (800 *g*, 10 min) on Ficoll-Hypaque (Pharmacia) and the mononuclear cell band was collected. Adherent cells were removed by a 1 hr preincubation in plastic culture flasks in RPMI containing 10% foetal calf serum (FCS). Cell loss was approximately 20% and contamination of recovered lymphocytes was < 2% monocytes as judged by phagocytosis of latex beads or antibody-coated erythrocytes. Culture medium consisted of RPMI 1640 containing 10% FCS, 50 μg/ml gentamycin and 5 mM HEPES buffer.

In some experiments, lymphocytes were rosetted with neuraminidase (Sigma)-treated sheep red blood cells (SRBC; 0·1 μl/ml, 2% SRBC, 30 min, 37°) and the rosetting cells (E^+) were depleted by centrifugation on Ficoll-Hypaque (1·077 gm/cm^3). The recovered E^- cells (10%–15%) were < 5% E^+ upon re-rosetting.

Antibody-dependent cell-mediated cytotoxicity (ADCC)

Lymphocytes were tested in 4 or 18 hr ^{51}Cr-release assays against relatively low NK-sensitive targets (Daudi, Chang, P815, RBL-5), which had been pretreated for 1 hr at 37° with a predetermined optimum concentration of antibody. RBL-5 was pretreated with a 1/10 dilution of rabbit anti-mouse thymocyte serum. Daudi, which expresses surface IgM, was pretreated with 1/50 rabbit anti-human IgM (kappa) serum; Chang and P815 cells were pretreated with 1/200 and 1/500 hyperimmune rabbit anti-Chang or anti-P815 serum, respectively. In some experiments, mononuclear cells were tested for ADCC activity in an 18 hr assay against ^{51}Cr-labelled human erythrocytes pretreated with 1/20 rabbit IgG anti-human RBC antibody.

Lectin-induced cell-mediated cytotoxicity

Purified lymphocytes were tested in an 18 hr ^{51}Cr-release assay against the low NK-sensitive target (P815-2) in the presence of a non-toxic concentration (2·0 μg/ml) of PHA-P (Wellcome) at a 1:1 E:T ration.

Cytolytic assays

Target cells (5×10^3) which had been labelled for 1 hr with ^{51}Cr (sodium chromate) were placed together with varying numbers of effector cells in 0·2 ml microwells (Linbro) in triplicate samples. The microplates were spun at room temperature for 5 min at 1000 r.p.m. and then incubated for 4 or 18 hr at 37°. The supernatant was measured in a γ counter. Autolysis was determined by culturing ^{51}Cr-labelled targets alone in the absence of effector cells and total label was determined by counting an aliquot of targets after resuspension in the microwells. The following formula was used to compute percentage lysis: % lysis = [(test c.p.m. − autolysis c.p.m.)/(total c.p.m. − autolysis c.p.m.)] × 100.

Lytic units

Lytic units (LU) were defined as the number of lymphocytes required to lyse 10% of the target cells, calculated according to the formula $y = A\ (1 - e^{-kx})$, where y is fractional ^{51}Cr-release, A is maximum cell-mediated lysis, k is a constant and x is the lymphocyte:target cell ratio as described by Pross, Baines, Rubin, Shragge & Patterson (1981). Relative NK activity was expressed as LU/10^7 cells and data pooled from several donors were analysed on the basis of arithmetic means, as shown, or geometric means (not shown) with similar results.

Mixed lymphocyte cultures

Raji cells, an Epstein-Barr Nuclear Antigen positive B-cell line derived from a Burkitt's lymphoma (HLA, A3, A27, B18, B35) were treated for 45 min at 37° with 100 μg/ml mitomycin C and used as stimulators. Peripheral blood mononuclear cells (not depleted of monocytes) were cultured in 2 ml Linbro wells at a concentration of 10^6/ml and Raji cells were added to yield a responder:stimulator ratio of 2:1. On day 3 or 6 of culture the cells were resuspended and transferred in 0·2 ml to microwells (2×10^5/well) and pulsed with 1

μCi of [^{3}H]-thymidine/well for 18 hr. The cells were harvested on glass fibre filter paper using a MASH harvester and counted in an LKB liquid scintillation counter.

Phagocytosis

Ox erythrocytes (1%) were incubated for 30 min at 20° with 1/200 hyperimmune rabbit anti-ox red blood cell antibody and washed three times. Peripheral blood mononuclear cells at a concentration of 5×10^6/ml were mixed with 2% antibody-coated ox RBC and centrifuged at 200 ***g*** for 5 min at 20° and then gently resuspended. The suspension was incubated for 18 hr at 37° and then treated for 5 min with Tris-ammonium chloride to lyse extracellular erythrocytes. An equal volume of crystal violet was added and the number of cells ingesting erythrocytes was determined after counting 400 cells under a light microscope.

Statistical method

A two-tailed Student's t test was used.

RESULTS

Natural killer (NK) cell function

Blood lymphocytes from all six CH patients had lower cytolytic activity against K562 target cells in 4-hr assays than did normal donors (Table 1). One of the normal donors was a sibling of one of the two CH patients of similar age and sex, and her level of cytotoxicity was within the normal range. NK activity was ten- to 100-fold less in CH patients than normals for lysis of several different target cell lines (K562, Molt-4, HL-60, YAC). The NK defect in CH lymphocytes was detected even in prolonged (18 hr) cytolytic assays although it was less (five-fold) whichever target cell lines were used, including K562, Daudi, Chang and P815.

Long term (18 hr) NK-mediated cytolysis of K562 cells was inhibited in both normal and CH patients by IgG-coated, but not uncoated, ox erythrocytes, which suggests that the effector in both normals and CH patients is FcR^+ (Fig. 1). In addition, removal of T cells forming rosettes with sheep erythrocytes, (E^+) did not reduce cytotoxicity by either normal or CH lymphocytes, indicating that at least 40%–50% of the effectors are E^-.

Antibody-dependent cell-mediated cytotoxicity

Blood lymphocytes from all six CH patients were markedly impaired (ten- to fifty-fold less) in their capacity to lyse antibody-coated Chang cells, Daudi, P815 or RBL-5 in short term (4 hr) assays (Table 2). These targets are resistant to NK-mediated cytolysis in a 4-hr assay. ADCC, after prolonged incubation (18 hr), was also defective as in the case of NK activity but less so (five- to twenty-fold). In contrast, cytolysis of antibody-coated human erythrocytes was not

Table 1. NK activity against a panel of target cells

Target	Assay time (hr)	Normal donors			CH patients			
		Lysis (%)	LU/10^7	N*	Lysis (%)	LU/10^7	N*	*P* value
K562	4	50±5†	1280±301‡	10	6±1	16±9	6	<0·005
Molt-4	4	25±5	488±168	4	7±2	32±23	4	<0·01
HL-60	4	16±5	154±123	3	1±1	0	2	
YAC	4	9±1	12±0	2	2±1	0	2	
K562	18	52±7	1089±294	9	19±8	190±33	6	<0·005
Daudi	18	60±9	800±200	3	8±1	168±33	3	
Chang	18	33±12	775±250	4	12±3	145±64	4	<0·05
P815	18	39±12	1100±264	3	13±4	175±43	4	<0·01

Blood mononuclear cell (depleted of monocytes) cytolysis of various tumour cell lines in a 4 or 18 hr ^{51}Cr-release assay. Mean values for each donor, tested on two–four occasions, over a 1 year period.

* Number of donors tested.

† Mean % lysis±SE for all donors tested at a 40:1 effector:target ratio in triplicate wells.

‡ Mean±SE number of lytic units/10^7 blood mononuclear cells.

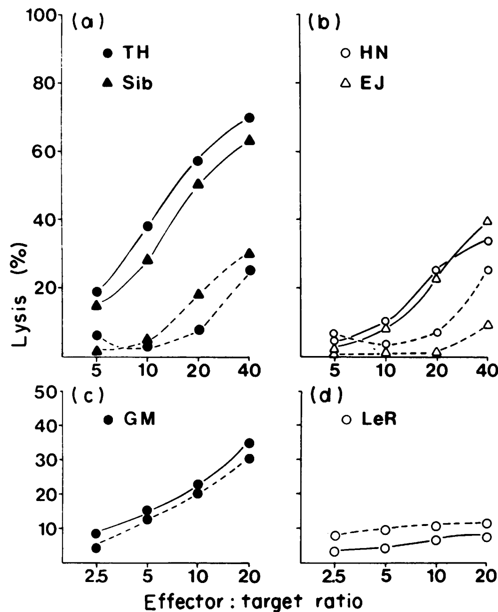

Figure 1. Blood mononuclear cell (depleted of monocytes) cytolysis of Fc receptor negative K562 cells in an 18 hr ^{51}Cr-release assay. (0△) CH patients; (●▲) healthy subjects. Ox erythrocytes (0·25%) coated with rabbit anti-ox IgG (- - - - -), or uncoated (——), added directly to the assay, (a and b). The effect of removing cells rosetting with sheep erythrocytes (E^-) (- - - - -) or unfractionated (——) before assay (c and d). Representative experiments; each value is the mean lysis (%) in triplicate wells.

Table 3. Lectin-dependent cytolysis

Normal donors		CH donors	
Donor	Lysis (%)	Donor	Lysis (%)
D.M.	28±8	J.C.	28±6
J.R.	26±4	H.N.	22±5
T.H.	20±2	E.J.	24±2
Sib.	27±3	W.P.	19±2
L.L.	25±2	La.R.	11±3
L.B.	35±5	Le.R.	30±2
J.W.	19±1		
B.S.	28±3		
G.B.	25±4		
Mean SE	26±2		22±3

Mean values±variation for each donor, tested on two–three occasions. The difference between the two groups is not significant ($P>0·05$).

significantly different in normals and CH patients. Cytolysis of red cell targets is mediated by different cells and by a different mechanism (Nelson, Bundy, Pitchon & Blaese, 1976).

Lectin-induced killer cells

Lectin-dependent cytolysis of tumour cells by blood

Table 2. ADCC activity against a panel of target cells

Target	Assay time (hr)	Normal donors Lysis (%)	LU/10^7	N*	CH patients Lysis (%)	LU/10^7	N*	P value
P815	4	47±5†	1075±160‡	4	6±2	30±19	4	<0·005
Daudi	4	36±5	857±239	7	9±4	62±39	5	<0·005
Chang	4	47±6	1089±220	9	9±3	36±14	6	<0·005
RBL-5	4	50±12	1867±706	3	24±8	136±43	3	
P815	18	84±6	1912±467	4	23±16	103±58	3	<0·01
Daudi	18	77	12,800	1	26±13	1700±1500	2	
Chang	18	72±6	2740±540	5	31±7	467±176	3	<0·01
HuRBC	18	24±4	300±58	4	21±87	260±87	4	NS§

Blood mononuclear cell (depleted of monocytes) cytolysis of various tumour cell lines in a 4 or 18 hr ^{51}Cr-release assay. Mean values for each donor, tested on two–four occasions, over a 1 year period.

* Number of donors tested.

† Mean % lysis±SE for all donors tested at a 20:1 effector:target cell ratio.

‡ Mean±SE number of lytic units/10^7 blood mononuclear cells. A lytic unit was defined as the number of effector cells required for 10% cytolysis.

§ NS, not significant ($P>0·05$).

Table 4. MLC proliferative response

	Normal donors			CH donors		
	Donor	c.p.m. × 10^{-3}	SI*	Donor	c.p.m. × 10^{-3}	SI*
A. Day 3	D.M.	42·8	20·5	J.D.	63·0	37·6
	J.R.	24·8	11·5			
	Mean ± SE	34 ± 9	16 ± 4		63	38
B. Day 6	L.B.	12·8	3·3	E.J.	15·3	2·6
	L.L.	21·3	2·2	H.N.	18·5	2·2
	D.M.	16·8	7·9	J.C.	25·6	15·3
	J.R.	20·6	9·7			
	Mean ± SE	18 ± 2	6 ± 2		20 ± 3	7 ± 4

Values represent the mean of duplicate cultures. The day 6 assay was repeated twice with similar results.
SI, stimulation index.

lymphocytes of normals and CH patients did not differ significantly ($P > 0{\cdot}05$; Table 3).

Mixed lymphocyte culture

The MLC response, a T-cell function (Dupont, Hansen & Yunis, 1976), of blood mononuclear cells from normal and CH patients did not differ greatly when stimulated by Mitomycin C-treated Raji cells and measured on day 3 or day 6 of culture ($P > 0{\cdot}05$; Table 4).

Phagocytosis

Monocyte phagocytosis of IgG-coated ox erythrocytes did not differ significantly in normals (3%–6%) and CH patients (4%–9%).

DISCUSSION

The data confirm that NK and ADCC activity against tumour cells is markedly impaired in patients with the Chediak-Higashi (CH) syndrome in short term assays (Roder *et al.*, 1980). The defect is also detected in prolonged assays, although it is less marked at 18 hr than at 4 hr. The lysis by CH lymphocytes in the 18 hr assay was mediated by FcR^+, E^- cells, as usual for NK cells.

ADCC activity against several tumour cell lines pretreated with antibody was also severely depressed in all six CH patients in both short and long term assays. ADCC against erythrocyte targets, a function of monocytes (Nelson *et al.*, 1976), was not significantly different in CH patients compared with controls. Lectin-dependent cytolysis of P815, a relatively NK-insensitive target, was normal in five out of six Ch patients; this is a function of cytotoxic T cells (Nelson *et al.*, 1976). The MLC proliferative T-cell response was normal in these CH patients, and others have shown normal MLC generated CTL function as well as normal MLC proliferative responses in three other CH patients (Griscelli, Durandy, Guy-Grand, Duguillard, Herzog & Prunieras, 1978). Delayed hypersensitivity to mumps, candida, dinitrochlorobenzene and streptokinase-streptodornase, and proliferative response to T-dependent mitogens are also normal (Blume & Wolff, 1972; Griscelli *et al.*, 1978; Gallin, Elin, Hubert, Fauci, Kaliner & Wolff, 1979). Beige mice, the animal model for the CH syndrome, show similar functions (Roder & Duwe, 1979).

NK function is also low in the X-linked lymphoproliferative syndrome (XLP), and some patients develop fatal EBV lymphomas (Sullivan, Byron, Brewster & Purtilo, 1980).

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